Impact of Urban TWP Contamination on CYP1A Expression in Endangered Redside Dace Populations in Canada and the United States

THE COLLEGE OF WOOSTER

Jordan Brillhart, Dr. Rebecca Williams; Department of Biochemistry and Molecular Biology

Abstract

Freshwater ecosystems are increasingly threatened by urbanization, which introduces contaminants through stormwater runoff. Tire wear particles release 6PPD-quinone (6PPD-q), a toxic transformation product linked to mortality in salmonids. The endangered Redside Dace (Clinostomus elongatus) may be vulnerable due to its reliance on clean, cool streams. This study measured environmental concentrations of 6PPD-q in Ohio and Ontario streams and evaluated CYP1A as a biomarker of exposure. Water samples were analyzed using LC/MS/MS, and CYP1A expression was assessed via protein extraction, SDS-PAGE, and Western blotting. Results showed no detectable 6PPD-q above 4 ng/L and limited CYP1A induction, suggesting episodic exposure and species-specific responses.

Background and Significance

- Freshwater ecosystems are highly impacted by urban pollution
- Tire wear particles (TWPs) release 6PPD-quinone (6PPD-q) into waterways

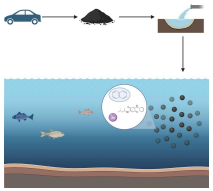


Figure 1. Vehicle tire abrasion produces particles that build up on roads, are mobilized by rain, and flow through storm drains into freshwater habitats. Fish encounter both the particles and the chemicals they carry

- 6PPD-q is highly toxic to fish at low concentrations
- Redside Dace is an endangered species sensitive to environmental change

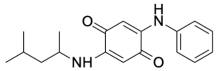
Objectives

1. Measure environmental concentrations of 6PPD-q
2. Determine if 6PPD-q induces CYP1A expression
3. Evaluate CYP1A as a biomarker in Redside Dace

Study System

- Species: Redside Dace (*Clinostomus elongatus*)

- Locations: Northeast Ohio + Ontario streams
- Contaminant: 6PPD-quinone (6PPD-q)



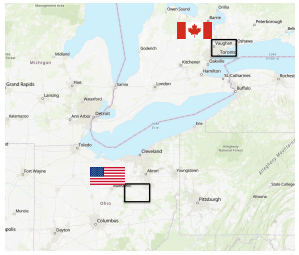


Figure 2. Sampling locations across the U.S.–Canada border region, including field sites in Northeast Ohio and the Greater Toronto Area.

Methods

Water Sampling — EPA Method 1634[4]

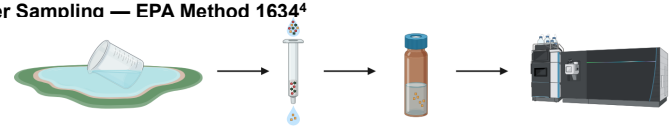


Figure 3. Overview of the sample-preparation process, beginning with environmental sample collection followed by solid-phase extraction (SPE) to retain analytes and remove particulates. SPE extracts are eluted into vials and subsequently analyzed using LC-MS to determine concentrations of chemicals of interest.

Fish Exposure — Protocol from Prior Toxicity Study[1]

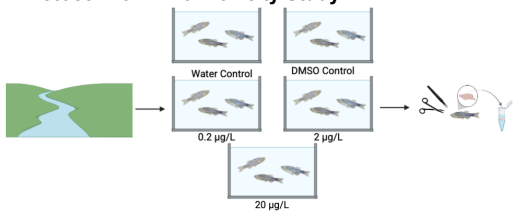


Figure 4. Experimental design showing Redside Dace exposed to five treatments: water control, DMSO control, 0.2 µg/L, 2 µg/L, and 20 µg/L. After exposure, fish were euthanized, dissected, and tissues collected for downstream chemical analysis

Protein Analysis:

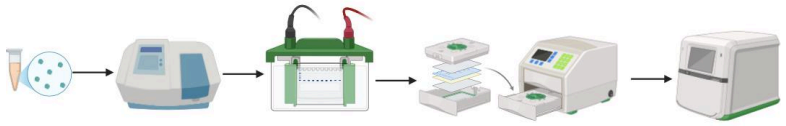


Figure 5. Workflow for Western blot analysis of Redside Dace tissues. Samples were homogenized and quantified prior to electrophoretic separation on SDS-PAGE gels. Proteins were transferred to membranes, probed with target antibodies, and visualized using an imaging system.

Results

Environmental Detection

- No 6PPD-q detected above LOD (4 ng/L) in any samples

CYP1A Expression

- CYP1A detected in positive control (~57 kDa)

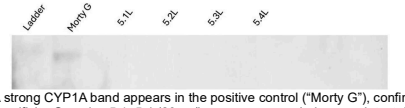


Figure 6. A strong CYP1A band appears in the positive control ("Morty G"), confirming antibody specificity. Samples 5.1–5.4 (20 µg/L exposure group) show no detectable CYP1A induction.

- Most samples showed no detectable expression
- Actin confirmed successful protein extraction

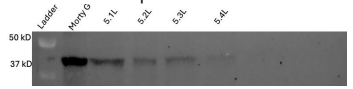


Figure 7. Actin bands are visible across all samples, including the 20 µg/L exposure group (5.1–5.4), confirming successful protein extraction and consistent loading for CYP1A analysis.

Discussion

- 6PPD-q is episodic, entering waterways primarily during precipitation-driven runoff
- Limited CYP1A induction may reflect:
  - Species-specific metabolic pathways
  - Low exposure concentration
  - Antibody sensitivity limitations

Future Work

- Conduct targeted 6PPD-q sampling during precipitation events
- Improve Western blot sensitivity by testing conjugated antibodies and evaluating additional Redside Dace tissues.

Acknowledgements

- Dr. Williams for mentorship and guidance
- The Copeland Fund for financially supporting this research
- Friends and cohort for making the IS fun and enjoyable
- My family, especially my mom, for unwavering their support
- Jesus Christ for strength and guidance throughout this work

Works Cited

1. Anderson-Bain, K., Roberts, C., Kohlman, E., Ji, X., Alcaraz, A. J., Miller, J., Gangur-Powell, T., Weber, L., Janz, D., Hecker, M., Montina, T., Brinkmann, M., & Wiseman, S. (2023). Apical and mechanistic effects of 6PPD-quinone on different life-stages of the fathead minnow (*Pimephales promelas*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 271*, 109697.
2. Canada. (2024). Species at Risk Act: Redside Dace (Clinostomus elongatus) profile. Government of Canada.
3. Chen, X., et al. (2023). *Oxidation of the tire rubber antioxidant 6PPD to 6PPD-quinone in the environment.*
4. EPA. (2024). *Draft Method 1634: Analysis of 6PPD-quinone in surface waters.* U.S. Environmental Protection Agency.